CERTAIN ASPECTS OF THE METABOLISM OF AMINO ACIDS

BY

LEONARD CHARLES SMITH

A.B., Montana State University, 1943

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
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I. THE ROLE OF INTESTINAL MICROORGANISMS IN THE SYNTHESIS OF THE NON-ESSENTIAL AMINO ACIDS

Introduction

In 1942, Welch (1), reported the antibacterial activity of succinylsulfathiazole, sold under the trade name of, "Sulfasuxidine". The extremely low toxicity, the poor absorption of the drug from the alimentary tract, and the pronounced effect of the compound on certain intestinal bacteria made it a very effective tool for the inhibition of synthesis of nutritional factors by intestinal bacteria. When Black *et al* (2), added this newly found drug to a regular synthetic ration, they found that the growth of rats was sharply reduced. Ransone and Elvehjem (3), showed that the reduced growth, occasioned by sulfasuxidine, could be effectively counteracted by adding folic acid and biotin to the diet. This indicated that these two compounds were being synthesized, at least in part, by intestinal bacteria. It was soon shown that many of the other vitamins necessary for normal growth are synthesized by intestinal microorganisms (4-6). The present study was undertaken with the purpose of determining whether intestinal microorganisms are involved in the synthesis of the non-essential amino acids in the albino rats.

Experimental

Weanling rats from the stock colony served as the experimental animals. Their rations consisted of casein, the ten essential amino acids, or nineteen amino acids each with and without sulfasuxidine. Preliminary experiments showed that, under these conditions, the regular vitamin supplements were not sufficient for optimum growth. When the vitamin supplements were raised, and folic acid was added, good growth was found to ensue.

Thirty-two male rats were then placed on a diet containing the nineteen amino acids. One half of the animals received a supplement of 1 per cent of sulfasuxidine, while the others did not. At the end of a period of twenty-eight days, the growth rates of the two groups were compared.

Forty-four male rats were then placed on a diet containing the ten essential amino acids. One half of the animals received sulfasuxidine at a 1 per cent level, while the remainder did not. The animals were allowed to grow on these rations for periods of twenty-eight days, and at the end of this time, the differences in growth rates were compared.

Summary

Diets containing casein, nineteen amino acids, or the ten essential amino acids, supplemented with the usual amounts of vitamins, were found to be incapable of supporting maximum growth when 1 per cent of sulfasuxidine was present in the food. When the vitamin supplement was increased, and folic acid was added, good growth was observed in the presence of the drug.

On a diet of nineteen amino acids, with the increased vitamin supplements and folic acid, there was practically no difference between the growth rates of the animals receiving the drug as compared with those not receiving it.

On a diet containing the ten essential amino acids, with the increased vitamin supplements and folic acid, there was a slight difference in the growth rate of the animals receiving the drug as compared to those not receiving it. When this difference was analyzed statistically, however, it was found to be of questionable significance.

Because of the findings outlined above, it may be concluded that if alimentary microorganisms play any role in the synthesis of the non-essential amino acids, the extent to which these reactions occur, is very small.

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II. GROWTH ON DIETS CONTAINING THE TEN ESSENTIAL AMINO ACIDS AT THEIR MINIMUM LEVELS WITH AND WITHOUT NITROGEN SUPPLEMENTS

Introduction

Experiments were begun in this laboratory several years ago to determine the minimum amount of each essential amino acid which is necessary for optimum growth. For this purpose, ten feeding trials were carried out. In each, one amino acid was fed in decreasing proportions while all the other components of the diet were held at constant and excess levels. The results of these experiments are shown in the table below.

The Minimum Amount of Each Essential Amino Acid Necessary to Support Normal Growth in the Rat when the Non-Essentials are Included in the Food

Amino acid	Per cent
Lysine	1.0
Tryptophan	0.2
Histidine	0.4
Phenylalanine	0.9
Leucine	0.8
Isoleucine	0.5
Threonine	0.5
Methionine	0.6
Valine	0.7
Arginine	0.2
	5.8

It then became a matter of considerable interest to know whether animals, fed a diet containing the ten essential amino acids at the minimum levels, could increase in weight, or even maintain their body weight. It would hardly be supposed that the animals could make the missing amino acids when their only source of nitrogen was that of the ten essentials furnished at the minimum levels. However, Rose and Womack (1), showed that not only maintenance, but slow growth resulted when the ten essential amino acids were fed at the levels indicated above. One possible explanation is that some of the products of cell catabolism, such as urea, ammonium ion, etc., may have been reutilized for the synthesis of the amino acids that were not included in the food. It seemed desirable, therefore, to feed diets containing the ten essential amino acids at the minimum levels, with and without nitrogen supplements, and compare the resulting growth rates.

Experimental

Male weanling rats from the stock colony served as the experimental subjects. The duration of the experiment in every case was twenty-eight days. In all cases where supplements were added, the total nitrogen of the diets was kept constant. The value selected was 1.57 gm. of nitrogen in 100 gm. of ration. This amount is found in the physiologically active amino acids of Mixture XXIII, a mixture commonly employed in this laboratory (*cf.* Rose, Oesterling, and Womack (2)). The supplements used were ammonium citrate, urea, glycine, and glutamic acid.

Summary

Comparative studies have been made of the growth of male weanling rats upon (1) a diet containing only the ten essential amino acids, each at the minimum level for optimum growth, and (2) similar diets supplemented respectively, with ammonium citrate, urea, glycine, and glutamic acid. The results demonstrate that animals receiving the basal diet are capable of gaining in weight at profoundly sub-normal rates. Doubtless this inhibition in growth is to be accounted for by the fact that, under the conditions specified, little nitrogen is available for the manufacture of the non-essential amino acids.

In contrast to the behavior of the animals upon the basal diet alone, the subjects which received any one of the nitrogenous supplements manifested much more rapid gains in weight. These findings demonstrate that ammonium citrate, urea, glycine, and glutamic acid are rather effective sources of nitrogen for the manufacture of the non-essential amino acids. Of the supplements employed, ammonium citrate and glutamic acid appear to possess comparable efficiency, and to be superior to either urea or glycine. Of the latter two compounds, glycine is more effective than urea.

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VITA

The author was born in Spokane, Washington, on January 31, 1921. He attended public schools there, and was graduated from North Central High School in June, 1938. In September, 1938, he entered Montana State University, and received the Bachelor of Arts degree from that school, with honors, in June, 1943. After one semester of graduate study at the University of Illinois, during which time he was a teaching assistant in inorganic chemistry, he began working full-time on a National Defense Research Committee contract under Dr. C. C. Price. In June, 1944, he went to Bushnell, Florida, to work on Chemical Warfare under the same contract. In February, 1945, he returned to the University of Illinois to work full-time on an anti-malarial program sponsored by the Committee for Medical Research under Dr. Price. In February, 1946, the author resumed graduate study and held a part-time teaching assistantship in inorganic chemistry. From June, 1947, until February, 1949, he was a part-time teaching assistant in biochemistry. He is joint author of the following publications:

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